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Increased endogenous interferon-gamma and neopterin correlate with increased degradation of tryptophan in human immunodeficiency virus type 1 infection

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1. Summary

Reduced tryptophan and increased kynurenine concentrations have been reported in patients with human immunodeficiency virus type 1 (HIV-1) infection. From in vitro data it appears that activated indoleamine 2,3-dioxygenase (IDO) is involved in this metabolic change. IDO is inducible by interferon-(IFN)-γ. We compared serum concentrations of IFN-γ and neopterin (the biosynthesis of which is also inducible by IFN-γ) with serum tryptophan and kynurenine of 42 patients with HIV-1 infection.

IFN-γ, neopterin and kynurenine levels were significantly increased compared to HIV-1 seronegative controls whereas tryptophan was significantly decreased. Various significant correlations were found between tryptophan, kynurenine, IFN-γ and neopterin concentrations. Highest degree of correlation was found between neopterin, IFN-γ and the kynurenine per tryptophan quotient which is the ratio between the product and the substrate concentration of IDO.

The data indicate that decreased tryptophan in HIV-1 seropositives may result from chronic immune activation and can be referred to increased activation of IDO.

Key words: HIV-1; Indoleamine 2,3-dioxygenase; Interferon-gamma; Neopterin; Tryptophan

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2. Introduction

Progressive infection with HIV-1 is associated with substantial loss of immune function. Diminished capacity of immune cells to respond to infectious agents renders patients susceptible to severe secondary infections, which finally cause the acquired immune deficiency syndrome (AIDS) and death [1]. Despite this, patients with HIV-1 infection frequently show multiple signs of chronic immune activation such as the presence of circulating immune complexes [2], of acid-labile interferon-(IFN)-α [3], of a soluble form of interleukin-2 (IL-2) receptor [4, 5], and of increased neopterin concentrations in body fluids [5–7]. Also, circulating IFN-γ is increased in patients with HIV-1 infection compared to healthy HIV-1 seronegative controls [8]. A significant positive correlation between IFN-γ and neopterin concentrations was found in patients [8].

We described reduced concentrations of tryptophan and increased kynurenine levels in patients with human immunodeficiency virus type 1 (HIV-1) infection [9]. It appears possible that activated indoleamine 2,3-dioxygenase (IDO) is involved in this metabolic change. IDO cleaves tryptophan to form *N*-formylkynurenine, and the enzyme is inducible by IFN-γ [10].

We were interested, whether an association existed

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(5)

between endogenous release of IFN-γ and changes of tryptophan metabolism in HIV-1 seropositives.

In this study, we compared IFN-γ and neopterin concentrations with tryptophan and kynurenine changes in 42 patients with HIV-1 infection.

3. Patients and Methods

Forty-two HIV-1 antibody-seropositives were included in this study (40 males, 2 females). They were aged 21–54 years (median 37.5 years). Twenty-eight were homosexuals, 6 intravenous drug users, 5 had both risk factors of HIV-1 exposure; for 3 patients, no apparent risk criteria could be established. According to the Walter Reed staging classification system (WR), 6 patients were WR2, 2 were WR3, 3 were WR4, 9 were WR5 and 21 were WR6. For one patient no $CD4^+$ T cell counts were available. He did not suffer from AIDS. When comparing laboratory results of patients at different WR stages, the patient was included in group WR2-4. Sixteen patients (all WR5 or 6) were under regular treatment with Zidovudine (azidothymidine, AZT).

IFN-γ concentrations were quantified by radioimmunoassay (Centocor, Malvern, PA, U.S.A.). The sensitivity of the test was enhanced by employing an optimized assay procedure allowing the detection of IFN-γ in sera with sufficient sensitivity to define normal range of healthy HIV-1 seronegative controls [11]. Beads with monoclonal anti-human IFN-γ antibody were incubated with 200 μl serum at room temperature for 16 h. Then the beads were washed with 3 ml of distilled water and incubated for a further 16 h with 200 μl of ^{125}I-labeled tracer. Radioactivity was counted with a gamma counter (Clini Gamma 1272; Wallac Oy, Turku, Finland). IFN-γ activity is expressed as NIH units. The detection limit of the test is 18 U/l. IFN-γ concentrations of the patients were compared to healthy HIV-1 seronegative controls [8, 11].

Serum neopterin concentrations were measured using radioimmunoassay (RIAcid, Henning-Berlin, Berlin, F.R.G.). Serum (50 μl) was incubated with 100 μl neopterin antiserum for 1 h at room temperature. Then 100 μl of ^{125}I-labeled tracer was added, followed by incubation for 1 h. Aqueous polyethylene glycol 6000 solution (2 ml; 60 g/l) was added. After centrifugation at 2000 × *g* for 10 min, radioactivity was counted using the gamma counter. The detection limit was 1 nmol/l. Neopterin concentrations of the patients were compared to those of healthy HIV-1 seronegative controls [12].

Tryptophan and kynurenine concentrations were measured by reverse-phase high pressure liquid chromatography as described using on-line deproteinization of samples without precipitation [13]. Tryptophan was measured by fluorescence detection at 285 nm excitation wavelength and 365 nm emission wavelength; kynurenine was measured by UV-absorption of 360 nm wavelength [14]. Tryptophan and kynurenine concentrations of patients were compared to healthy HIV-seronegative controls [14].

Statistical evaluation of grouped data was done employing the Wilcoxon rank test. Correlation analysis was done using Spearman's rank correlation coefficients. The results were confirmed by computing linear regression coefficients (data not shown).

TABLE 1

Serum IFN-γ, neopterin, tryptophan and kynurenine concentrations in 42 HIV-1 seropositive patients compared to healthy HIV-1 seronegative controls [1, 10, 12]

	HIV-1 seropositive patients			HIV-1 seronegative controls
	mean ± S.E.	median	25th – 75th percentile	mean ± S.E. (n)
IFN-γ (U/l)	259 ± 70*	102	57 – 233	23.5 ± 1.7 (76)
Neopterin (nmol/l)	24.6 ± 2.8*	19.1	14.9 – 28.8	5.34 ± 0.14 (359)
Tryptophan (μmol/l)	57.0 ± 2.6*	57.0	41.6 – 72.0	91.0 ± 6.63 (11)
Kynurenine (μmol/l)	3.45 ± 0.14*	3.20	2.80 – 3.92	2.31 ± 0.23 (11)

*Significantly different from healthy HIV-1 seronegative controls ($P < 0.01$; Wilcoxon rank test).

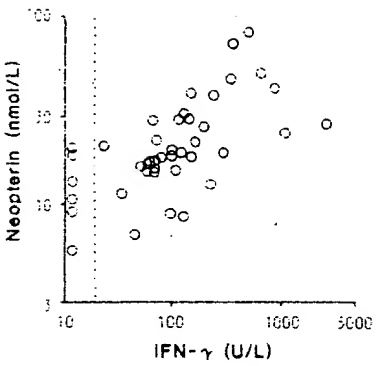

Fig. 1. Serum IFN-γ and neopterin concentrations (Spearman's rank correlation coefficient $r_S = 0.583$, $P < 0.001$). Dotted line shows the lower limit of detection for IFN-γ = 18 U/l.

4. Results

Thirty-six of 42 (85.7%) HIV-1-seropositives had detectable IFN-γ in serum. The IFN-γ concentrations were significantly higher compared to HIV-1 seronegative controls (Table 1); 23 (54.8%) had increased concentrations compared to the upper normal limit (95th percentile of HIV-1-seronegative blood donors = 100 U/l) [8, 11]. Patients also had higher neopterin and kynurenine concentrations in serum compared to healthy HIV-1 seronegative controls (Table 1). In contrast, tryptophan levels were lower in HIV-1 seropositives.

Within our patient group, serum neopterin and IFN-γ levels were higher in WR5/6 patients (mean ± S.E.M.; neopterin, 27.5 ± 3.05 nmol/l; IFN-γ, 312 ± 100 U/l) than in WR2-4 patients (neopterin, 18.7 ± 4.30, p = 0.011; IFN-γ, 178 ± 76.8, p = 0.092). Kynurenine was nearly identical in both groups (WR2-4, 3.4 ± 0.29 μmol/l; WR5,6, 3.5 ± 0.16). Serum tryptophan was slightly lower in WR5,6 patients (54.1 ± 3.0 μmol/l) than in WR2-4 patients, but the difference was not significant (61.5 ± 6.03; $P = 0.15$).

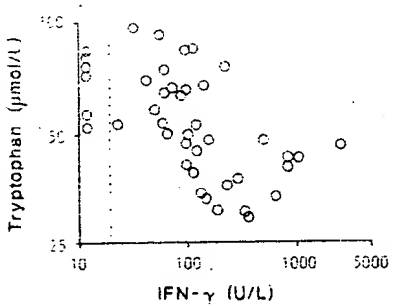

Fig. 2. Correlation between serum IFN-γ and tryptophan concentrations in 42 HIV-1 seropositive individuals (Spearman's rank correlation coefficient $r_S = -0.0616$, $P < 0.001$). Dotted line shows the lower limit of detection for IFN-γ = 18 U/l.

TABLE 2

Associations (Spearman rank correlation coefficients and P values) between serum IFN-γ, tryptophan, kynurenine and neopterin concentrations and kynurenine:tryptophan ratios in 42 HIV-1 seropositive patients.

	Tryptophan	Kynurenine	Neopterin
IFN-γ	$r = -0.616$	$r = 0.114$	$r = 0.583$
	$P < 0.001$	$P > 0.1$	$P < 0.001$
Tryptophan		$r = 0.040$	$r = 0.716$
		$P > 0.1$	$P < 0.001$
Kynurenine			$r = 0.411$
			$P = 0.008$

Several significant correlations were seen between the variables studied (Table 2). Serum IFN-γ concentrations correlated positively with serum neopterin levels (Fig. 1) and negatively to serum tryptophan levels (Fig. 2). No correlation existed between IFN-γ and kynurenine concentrations (Table 2) whereas a significant correlation was found between serum IFN-γ and the kynurenine:tryptophan ratio ($r = 0.615$, $P < 0.001$). Serum neopterin levels correlated positively to kynurenine concentrations (Fig. 3) and to the kynurenine:tryptophan ratio ($r = 0.842$, $P < 0.001$).

No difference was observed with respect to IFN-γ, neopterin, tryptophan, and kynurenine levels in pa-

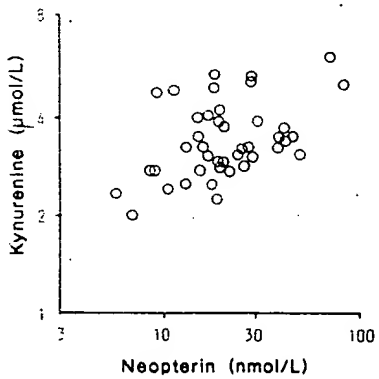

Fig. 3. Correlation between serum neopterin and kynurenine concentrations (Spearman's rank correlation coefficient $r_S = 0.411$, $P < 0.008$).

tients either treated or not treated with AZT. However, during follow-up of patients when treatment with AZT was initiated, we observed decreasing IFN-γ, neopterin and kynurenine concentrations; in contrast, tryptophan levels increased (data not shown).

5. Discussion

Reduced capacity of T cells to respond to antigenic and mitogenic stimulation in vitro is frequent in patients with HIV-1 infection. Nearly all patients with advanced stages of infection present with severe immune deterioration [1]. T cells of these patients show almost diminished or absent production of cytokines such as IL-2 and IFN-γ upon stimulation [1, 15]. This in vitro finding agrees with partial or complete skin test anergy of patients. In contrast, circulating IFN-γ concentrations are increased in approximately half of our patients with HIV-1 infection compared to healthy HIV-1 seronegative controls. This result confirms earlier data [8]. Also Murray et al. found similar concentration ranges of IFN-γ in HIV-1 seropositives using radioimmunoassay [15].

Our data further show that IFN-γ is biologically active in HIV-1 seropositives. Significantly decreased tryptophan levels found simultaneously with increased kynurenine levels in our patients indicate active degradation of tryptophan via induction of IDO. The expression of IDO as well as activation of the enzyme can be induced by IFN-γ [10]. In addition, IFN-γ correlates positively to neopterin concentrations. Increased neopterin levels reflect induction of GTP-cyclohydrolase I (EC 3.5.4.16), which is strongly inducible by IFN-γ in vitro [12].

The decrease of tryptophan concentrations is much stronger than the increase of kynurenine in our study, and the correlations between IFN-γ and neopterin are much stronger with tryptophan than with kynurenine. This can be explained by the fact that kynurenine once produced is rapidly metabolized. The tryptophan metabolite quinolinic acid was found to be increased during the course of HIV-1 infection [16]. In agreement with this, IDO is induced by IFN-γ; the subsequent enzymes downstream the tryptophan degradation pathway are constitutive in various human cells [17].

Changes of tryptophan concentrations were found to correlate to neurological/psychiatric symptoms in patients with HIV-1 infection [14]. Enhanced degradation of the essential amino acid tryptophan may be the reason for this observation: (1) Reduced formation of the neurotransmitter 5-hydroxytryptamine [18], and (2) increased formation of neurotoxic tryptophan metabolites such as quinolinic acid may contribute to precipitation of symptoms in HIV-1 seropositives [16].

The finding of circulating IFN-γ in HIV-1 seropositives could be also relevant with respect to the pathogenesis of AIDS. The presence of IFN-γ indicates that T cells of patients with HIV-1 infection are chronically stimulated. Infection with HIV-1 itself or by secondary pathogens may be responsible for immune stimulation which in turn may induce and support replication of HIV-1 in patients [5, 19]. Our data demonstrate that (1) IFN-γ is increased in HIV-1 infection and (2) metabolic changes can be detected which can be referred to the activity of IFN-γ. There exists an obvious discrepancy to in vitro findings on IFN-γ. However, this observation is not unique to HIV-1 infection. In several other diseases which are associated with chronic immune activation such as graft versus host disease, autoimmune disorders and other chronic infections, reduced in vitro responsiveness of peripheral blood mononuclear cells is found [20].

Acknowledgements

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